reading of the application. Claims 1-8 and 18-24 are pending in the application. Claims 9-17 are withdrawn currently from consideration. Claims 18, 19 and 21-24 are amended herein.

Outstanding Issues:

- The specification has been objected to and all claims have been rejected under 35 U.S.C § 112, first paragraph.
- Claims 21-24 stand rejected under 35 U.S.C. § 112, second paragraph.
- Claims 1-8 and 18-24 stand rejected under 35 U.S.C. § 102(a) as being anticipated by Chamberlain et al.
- Claims 1-8 and 18-24 stand rejected under 35 U.S.C. § 103 as being unpatentable over Kogan et al.

35 U.S.C. § 112, First Paragraph

The Examiner objects to the specification and rejects all claims under 35 U.S.C. § 112, first paragraph, for failing to provide an enabling disclosure. Specifically, the Examiner contends that Applicants added new matter in their Response of September 1991 by reciting that all primers in a reaction have similar melt characteristics. The Examiner makes this contention despite agreeing with the Applicants that amending Table 1 of the specification to include melt temperature values for the hybridization between the primers and their complements is not new matter. In their Response of August 24, 1994 regarding the predecessor of the current application, Applicants argued that Table 1 not only discloses melt temperatures inherent to primers and their complements, but discloses an inherent *range* of melt temperatures, and one skilled in the art would recognize the significance of the range. Aside from the values disclosed in Table 1, Applicants provided support for this issue at page 16, lines 15 to 34, and specifically 25-27 of the specification, which reads, "[t]he temperature is dependent on the length, the uniqueness of the primer sequence and the relative percentage of GC

bases." In the present Response, Applicants provide additional verification that a melt temperature range for primers is disclosed in the application.

Attached hereto are Declarations from respected scientists from around the world, namely Drs. Umadevi Tantravahi, Director of Molecular Genetics and Cytogenetics in the Department of Pathology and Laboratory Medicine at Women & Infants Hospital of Rhode Island; Hugo Barrera-Saldaña, Chair of the Department of Biochemistry at Universidad Autonoma de Nuevo Leon in Monterrey, Mexico; Andres Metspalu, Professor at the Institute of Molecular and Cell Biology at Tartu University, in Tartu, Estonia; Lennie Pineda, Director of the Unidad De Genetica Medica at the Universidad Del Zulia in Maracaibo, Venezuela; Gert-Jan van Ommen, Head of the Department of Human Genetics at the Medical Genetics Center in Leiden, the Netherlands; Francesco Salvatore, Full Professor of Human Biochemistry at the Dipartimento di Biochimica e Biotecnologie Mediche, Sezione di Biochimica e Biologia Molecolare in Naples, Italy; and Dr. Julian Gordon, Senior Research Fellow of the Volweiler Society of Abbott Laboratories. Please note that the Declarations of Drs. Umadevi Tantravahi, Hugo Barrera-Saldaña, Andres Metspalu, Lennie Pineda, Gert-Jan van Ommen, and Francesco Salvatore state that the DMD protocol and kits provided by Dr. Caskey, and the results obtained therewith, led these investigators to design other multiplex PCR tests. Dr. Gordon states that the protocol provided to the above investigators and the protocol disclosed and claimed in the patent application are the same. As additional evidence on this point, Dr. Gordon provides a side-by-side comparison of the procedures.

Applicants assert that in order for tests drawn to other genes to have been developed using the DMD protocol, the investigators must have recognized the inherent primer/complement melt value range in the protocol, as primer/complement melt value is one of the most important parameters in performing multiplex amplification successfully (*see* discussion under 35 U.S.C. § 103 *infra*). Additionally, the Declaration of Dr. Julian Gordon states that, in his technical opinion, a person of ordinary skill in the art would recognize that the present specification teaches the use of primers having similar melt temperatures. Thus, Applicants contend that the melt value range for primer/complement combinations disclosed in the application of Caskey et al. is

inherent, and that those skilled in the art perceive and understand the inherency from reading the specification.

New matter does not include that which was inherently disclosed in the original disclosure, *In re Nathan*, 328 F.2d 1005, 1008-09 (CCPA 1964). Further, the invention claimed in an application does not have to be described in *ipsis verbis* in order to satisfy the description requirement of § 112. *Ralston Purina Co. v. Far-Mar Co.*, 772 F.2d 1575, 1578 (Fed. Cir. 1985). For a disclosure to be inherent, *it must lead one skilled in the art to the critical limitation. Id.* (emphasis added). Clearly, the disclosure of the present invention satisfies this criterium.

Thus, Applicants insist that not only are the melt *values* inherent to the specification, but the *range* of these values is inherent as well. Similarly, for claims 18 and 19, the melt values are inherent, thus so is the range. As for what range is disclosed, Applicants concur that the range is 8.3°C, for the highest and lowest melting points of the primers disclosed and the claims have been amended to reflect this value. Further, the value of 4.4 °C is determined in the following way: the first primer of sets b, d and g and the second primer of sets c, e and f have the lower Tms of the primer pairs disclosed. Calculating the temperature range using only these lower Tms, reveals a range of no more that 4.4 °C. Again, the claims have been amended herein to reflect precisely the ranges disclosed in the specification.

In view of the Declarations enclosed, the amendments made to the claims and the remarks above, Applicants respectfully request reconsideration of all claims, and withdrawal of the rejection of the claims under 35 U.S.C. § 112, first paragraph.

35 U.S.C. § 112, Second Paragraph

Claims 21-24 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the invention. Specifically, the Examiner states that the claims are vague as there is no citation that relates back to the preamble detection practice of the claims. Claims 21-24 have been

amended herein so that the last step of the claims relates back to the purpose of the method stated in the preamble.

In view of the amendments made herein, Applicants request respectfully reconsideration of claims 21-24 and withdrawal of the rejection of the claims under 35 U.S.C. § 112, first paragraph.

35 U.S.C. § 102 (a)

The Examiner has rejected all claims under 35 U.S.C. § 102 (a) as being anticipated by Chamberlain et al., *1988 Am. J. or Human Gen.*, Vol. 43, Abstract 0711. Applicants respectfully traverse this rejection.

Enclosed please find the Declaration under 37 C.F.R. § 1.132 of Dr. Jeffrey Chamberlain. In his Declaration, Dr. Chamberlain states that Nancy Farwell worked in the capacity of a research technician, and merely assisted the inventors with their experiments. Ms. Farwell was not involved in the planning of experiments or interpreting data, nor was she involved in establishing multiplex PCR. As a research technician, she helped only in screening a genomic library to isolate gene fragments which were used ultimately by the inventors to establish the invention. Thus, the *Am. J. of Human Genetics* abstract is not a proper reference under 35 U.S.C. § 102(a) as the invention was not known or used *by others* in this country. *See, e.g., In re Katz*, 687 F.2d 450 (CCPA 1982).

In view of the amendments made herein, Applicants request respectfully reconsideration of all claims and withdrawal of the rejection of the claims under 35 U.S.C. § 102 (a), first paragraph.

35 U.S.C. § 103

The Examiner has rejected all claims under 35 U.S.C. § 103 as being unpatentable over Kogan et al. The Examiner argues that the present invention cannot be distinguished from Kogan by recitation that all primers in a reaction have similar melt characteristics, because this characteristic of the invention is considered to be new matter. Applicants contend that in light of the attached Declarations and the remarks

made above regarding the new matter rejection, the limitation that all primers have similar melt characteristics is not new matter. Therefore, the present invention is distinguishable from Kogan et al., and the rejection under § 103 is improper.

Additionally, Applicants request that the Examiner note that the Declarations of Drs. Barrera-Saldaña, Metspalu, Piñeda de Del Villar, van Ommen and Salvatore state that there was a need in the art for multiplex amplification using more than two sets of primers, since prior to the invention of Caskey et al., screening for a plurality of DNA sequences required a large number of separate assays for each sequence. None of the Declarants had performed multiplex assays prior to being provided with the materials and methods from Dr. Caskey. The investigators only developed additional tests after learning of the multiplex assay from Dr. Caskey. Thus, experts in the field saw an unmet need and found a solution to this need only after receiving the materials and methods from Dr. Caskey. These facts provide very strong evidence that the invention is non-obvious.

As for Kogan et al., the Examiner asserts that Kogan does not recognize problems with multiple primer usage. However, neither does Kogan et al. discuss any parameters that would allow multiple primers to be used successfully when amplifying multiple sequences. Kogan et al. do not recognize problems with multiple primers precisely because they did not realize the problems that would be encountered. This lack of awareness no doubt led them to believe there would not be any problems, and this is why it was necessary for the inventors of the present invention to have to work so hard to make the method work. The present invention was not developed based on the limited knowledge Kogan et al. disclosed--Kogan et al. did not recognize or anticipate problems, and as a result they were unable to suggest ways to overcome those problems.

The only data presented by Kogan regarding use of multiple primers is in Figure 1 of the manuscript, in which two sequences are co-amplified using four primers. A good copy of Kogan et al. is enclosed in which Figure 1 is reproduced very clearly.

However, Kogan et al. specifically point out that their reaction *is not specific*. The last sentence on the left column on page 987 of the article states "[a]lthough some background bands are present . . . ." Thus, Kogan et al. acknowledge explicitly that they were unable to achieve a specific reaction, even with only four primers in the reaction. They offer no explanation for this failure, nor do they offer any suggestions as to how to correct this problem, nor do they indicate that this problem could become increasingly problematic as more primer pairs are added to the reaction, nor do they offer suggestions as to how to overcome such problems should they be encountered.

In fact, a careful reading of the manuscript of Kogan et al. reveals that multiplex amplification is disclosed and discussed rather offhandedly--Kogan et al. certainly do not disclose suggestions for dramatic improvements in multiplex methods, particularly for the use of six or more primers. In the first paragraph of the results section (page 987), Kogan et al. do attempt to address the subject of specificity. They found that using Taq polymerase instead of Klenow polymerase was the key to their success. They state Taq is highly specific, although in the next sentence they contradict that point by saying, "the *vast majority* of the fragments are the correct size and sequence." Vast majority is not the same as all. Moreover, vast majority may be adequate when only two products are being sought, however, it is clearly not good enough if many products need to be specifically amplified and *interpreted*.

Kogan et al. go on to write (also in paragraph 1 of the Results section) that "[i]t appears that raising the temperature of the *reaction* mixture from 37 degrees (with klenow) to 63 degrees (with Taq polymerase) is responsible for this specificity" (emphasis added). Note several comments: first, "it appears" is speculative, and no evidence is presented to support this statement; and second, the phrase "temperature of the reaction mixture" indicates that Kogan et al. focus throughout the manuscript on the use of Taq polymerase and the higher temperature with which one does "the reaction." Kogan et al., however, fail to discuss the fact that PCRs are typically done at several temperatures which are cycled. Is it the annealing temperature (of the primers) they think is important, or is it the extension temperature of the polymerase which is so important? In fact, Kogan et al. argue that is the *extension temperature*, not the annealing temperature (Tm). As for Kogan et al.'s views on the importance of

the annealing temperature, one simply needs look at the Methods section on page 986 ("Sequence amplification with . . . "). In this section, at the top of the left hand column, Kogan et al. discuss their choice of annealing temperature. Here they stated that they anneal by using "*a 30 second cool-down at room temperature to allow primers to reanneal*" (emphasis added). However, later in the same sentence they specify exactly 63 degrees as their extension temperature. Not 64 degrees, not 62 degrees, but 63 degrees. However, a similar importance is not attached to the Tm of the primers. Nor is there any discussion of optimizing the annealing temperature (inherent to Tm) or of optimizing the primer design. Further in the manuscript, in the first paragraph of Results, Kogan et al. state that the reason their reaction worked so "nicely" is that at 63 degrees (as opposed to 37 degrees) "priming occurs only when the template and primer are exactly matched . . . ." Thus, Kogan et al. discuss offhandedly the annealing temperature, but claim that 63 degrees is the temperature at which the primers are perfectly matched. The teaching of the manuscript seems to be that as long as extending is done at precisely 63 degrees, multiplex PCR will be accomplished successfully. Kogan et al. suggest that as long as the extension was kept at precisely 63 degrees, all reactions would work pretty well and be "mostly specific."

Subsequently, however, Kogan et al. disprove themselves on this point. In the legend to Figure 2 (page 988) they write "[i]n this experiment, samples were amplified with primers 7.1 and 7.2, since amplification with these primers yielded clearer results than amplifications with the primers 7.7 and 7.10 shown in Figure 1." Thus, Kogan et al. acknowledge that their own conditions did not work well enough for the experiments they were describing in Figure 2. How did Kogan et al. use their knowledge of multiplex PCR to solve this dilemma? They made a new set of primers, but disclose no insight as to why the new primers were successful.

The Examiner on page 6 of the April 4, 1995 Office Action admits Kogan et al. were unable to achieve specificity in Figure 2, and had to switch to a new set of primers. However, the Examiner then states that such a switch was not necessary, as

"both sets of primers gave usable results." Therefore, "[t]he primer pairs may be interchanged . . . (and Kogan et al.) . . . do not recognize any problem with multiple primer usage." Applicants contend that this is contradictory. The authors state explicitly that one primer pair was not specific enough for them to use, yet the Examiner contends Kogan et al. did not recognize problems with multiplex amplification. Clearly Kogan et al. recognized problems with the specificity of their primers. Further, it must be remembered that authors typically show their best data in a manuscript, and if Kogan et al. felt there was enough of a problem that they must switch to a new primer pair, it may be there was more of a problem than was disclosed ultimately in the manuscript.

Most importantly, however, is the fact that the present invention considers a method that uses many primer sets--ones whose sequences are different from the ones used by Kogan et al. Nowhere in the manuscript by Kogan et al. is there any disclosure, teaching or suggestion as how one would overcome the limitations imposed by using more than two primer pairs. Presumably, this is because Kogan et al. did not attempt to use more than two primer pairs, nor were they aware that such a situation would cause problems. It was only when the inventors of the present multiplex method attempted to perform such a reaction did the problems with multiplex amplification become apparent, and why it was necessary to invent a new method to analyze these sequences in multiplex format. The Examiner states Kogan *et al.* did not recognize a problem, therefore there was not a problem. However, the enclosed Declaration under 37 C.F.R. § 1.132 by Dr. Jeffrey Chamberlain states clearly that having a narrow range of Tms for the primers is probably the single most important aspect of multiplex DNA amplification, that Tms are inherent with a primer, and choosing primers with similar Tms is critical to a successful multiplex reaction. This aspect of the multiplex amplification method was one which was clearly *unanticipated* by Kogan et al. and an aspect for which Kogan et al. were unable to offer guidance.

In addition to the above comments regarding the inappropriate application of the Kogan et al. reference, Applicants contend that the rejection of the claims under 35 U.S.C. § 103 is defective because *prima facie* obviousness has not been established. It is well-established that "[r]eferences relied upon to support a rejection under 35 U.S.C.

§ 103 must provide an enabling disclosure, *i.e.*, they must place the claimed invention in the possession of the public." *In re Payne*, 606 F.2d 303, 314 (CCPA 1979); citing *In re Brown*, 329 F.2d 1006, 1011 (CCPA 1964). Further, "[a]n invention is not 'possessed' absent some known or obvious way to make it." *Payne*, 606 F.2d at 314. As Kogan et al. do not provide an enabling disclosure for the practice of multiplex amplification for at least three pairs of primers, Kogan et al. is not a proper grounds for rejection under 35 U.S.C. § 103.

Applicants contend that in light of the attached Declarations and the above remarks, as well as those remarks made regarding the rejections under 35 U.S.C. § 112, first paragraph, above, the rejections under 35 U.S.C. § 103 have been overcome, thus, Applicants respectfully request reconsideration of all claims.

Applicants assert that in view of the Declarations enclosed and the above remarks, the application is now in condition for allowance. Accordingly, Applicants respectfully request that a letters patent be issued on the application. If any requirements remain, please contact the undersigned at (713)651-5325 for quick resolution.

Respectfully submitted,

Date: 10/3/95

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